I. 現在実現化に最も近い，外科領域における再生医療研究

3. 生体内組織再生誘導型の人工気管

Artificial trachea with in situ induction of tissue regeneration

1. 福島県立医科大学医学部耳鼻咽喉科学講座
2. 北野病院耳鼻咽喉科
3. 京都大学再生医科学研究所再生医学応用研究部門臓器再建応用分野

大森　孝一[1]・多田　靖宏[1]・野本　幸男[1]・谷　亜希子[1]
Koichi Omori（教授）　*Yasuhiro Tada*（講師）　*Yukio Nomoto*（講師）　*Akiko Tani*
仲江川雄太[1]・金丸　眞一[2]・中村　達雄[3]
Yuta Nakaegawa　*Shin-ichi Kanemaru*（主任部長）　*Tatsuo Nakamura*（准教授）

Summary

気管・輪状軟骨の再建は最も難しい外科治療のひとつである。筆者らは生体内組織再生誘導型の人工気管を開発し，気道としての枠組みを保持するためポリプロピレン製メッシュを管状にし，同素材のリングで補強し，その表面に組織再生の足場として医療用のブタ皮膚由来のコラーゲンスポンジを付着させた。

大型動物への移植実験で最長５年の経過観察で安全性，有効性が確認され，施設内倫理委員会の承認のもと，2002年より筆者らは生体内組織再生誘導型の人工気管を用いた気道の再生医療を世界に先駆けて行っている。甲状腺癌の気管浸潤例に対する気管再建と喉頭気管狭窄例に対する病変切除後の二期的再建を行い，成人11例において最長７年の経過観察で内腔上皮再生が得られている。

生体内組織再生誘導型の人工気管は製造方法が完成しており，製造から販売を取り扱う企業が決まれば医療機器としての実用化が実現し，患者のQOL向上に寄与できるものと思われる。

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Key Words　人工気管，生体内組織再生誘導，*in situ* Tissue Engineering

はじめに

気道は鼻腔，口腔，咽頭，喉頭，気管と気管支・細気管支によって構成される。喉頭は呼吸，嚥下，発声という重要な役割をもち，気管は呼気や吸気の通り道であると同時に，線毛運動と咳反射により分泌物や異物を除く排泄路として機能している。

気管や輪状軟骨に悪性腫瘍や狭窄性疾患を生じると，病変の切除後に気道を再建する必要がある[1)]。悪性腫瘍としては甲状腺癌，肺癌などがあり，狭窄性疾患としては内腔からの損傷と外

表１　気管・輪状軟骨再建の主な適応疾患

悪性腫瘍	甲状腺癌，肺癌，気管原発腫瘍
炎症性狭窄	気管内腔からの損傷（気管挿管チューブ，気管カニューレ，熱傷，化学的腐食剤）
	気管外からの損傷（交通外傷，刃物による裂傷，銃創，気管切開術後）
	炎症性疾患（再発性多発軟骨炎，結核）
	特殊な疾患（いわゆるWegener肉芽腫，アミロイドーシス）

◆メモランダム◆

in situ Tissue Engineering ―組織工学の新しいトレンド―

培養室のシャーレのなかで組織や臓器を作る組織工学（Tissue Engineering）は1990年代に始まり夢の技術として注目された。しかしながら，シャーレのなかで作られた組織を体に埋め込むと吸収されるという問題が明らかになった。そこで，再生医療の実用化に向けて，患者の体内の本来の場所で組織を再生させる*in situ* Tissue Engineeringが組織工学の新しいトレンドとして期待を集めている。

からの損傷があり，炎症性疾患もある（表1）。

気管・輪状軟骨を再建する際には，管腔を保持する硬度をもつ枠組みと内腔面に線毛をもつ粘膜を同時に再建することが理想的である。また，喉頭の下部を構成する輪状軟骨は気管より管腔が狭く再狭窄を起こしやすいことから，再建は難しい。これらの課題を解決するために，筆者らは生体内組織再生誘導型の人工気管を開発しており，現在実現化に最も近い，外科領域における再生医療研究として，その開発コンセプトと少数例の臨床経験を紹介する。

従来の気道再建外科

従来の気道再建外科を表2にまとめる。1970年代からGrilloのグループが気管端々吻合術を報告してきたが[2]，広範囲切除の限界，縫合不全や縦隔炎などの重篤な合併症，術後の頸部前屈姿勢など，容易な手術ではない。輪状軟骨切除後の端々吻合術も一部の施設で行われている。1980年代からCottonらにより移植をともなう再建術が報告されており[3]，硬性再建には骨，軟骨が用いられ，内腔面には皮膚や粘膜が用いられるが，自家遊離移植では複数部位や複数回にわたる手術侵襲が必要であるうえに，移植片の移動や吸収の問題があり気道の枠組みとしての長期的安定性を保持するのが難しい。人工材料にはシリコン，チタンなどの材料が用いられてきたが安定した成績を上げていない[4]。悪性腫瘍に対しては，進行甲状腺癌の気管浸潤例では十分な安全域をとった治癒切除が望まれるが，大きく切除すれば再建は難しくなり，自家遊離移植は欠損部の大きさによって制限される。

表2　従来の気道再建外科治療と問題点

	問題点
端々吻合 （気管，輪状軟骨）	縫合不全（吻合部緊張や血流不全），術後管理（長期の頸部前屈）
自己組織移植 （硬性組織として軟骨や骨，複雑な手術操作（複数部位，複数回） 内腔面として皮膚や粘膜）	
人工材料移植 （シリコン，チタン，ポリプロピレンメッシュ）	低い成功率

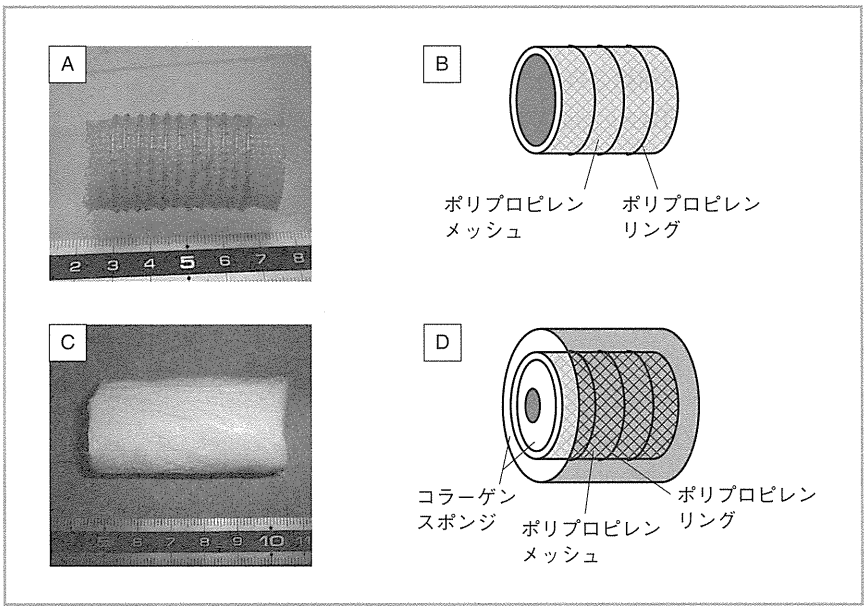

図1　人工気管

A：骨格，B：骨格のシェーマ，C：外観，D：外観のシェーマ

（カラーグラビアp3　写真5参照）

場の理論と生体内組織再生誘導型の人工気管

1993年，LangerとVacantiは体外で細胞を培養して工学的手法により臓器や組織に近いものを再生させる

Tissue Engineeringという概念を提唱したが[5]，体外で作られた組織が体内への移植後に吸収されるなどの問題があり，臨床応用へのハードルは高い。一方，生体内で組織の再生を誘導する手法は*in vivo* Tissue Engineering，生体内の本来の場所で組織の再生を誘導する手法は*in situ* Tissue Engineeringといわれる。

傷害された組織・臓器は，本来，自己再生能力を有しているが，生体組織が大きく欠損した場合や急激な組織修復により組織再生の場が奪われてしまうと，瘢痕化などにより元の組織が再生しない。組織再生の適切な場が適切な組織再生を促進するという考え方を場の理論という。1995年以降，中村らは場の理論に基づいたコンセプトで，コラーゲンスポンジを主体とした足場の移植で気管[6]，食道，胃，小腸，末梢神経などの組織再生を報告した。

生体内組織再生誘導型の人工気管（図1）は[6]，気道としての管状の枠組みを保持するため，ポリプロピレン製メッシュを管状にし，同素材のリングで補強した。ポリプロピレン製メッシュは特定保険材料として従来から胸壁や腹壁の補強に臨床で使われている。ポリプロピレン管の表面に，組織再生の足場としてコラーゲンスポンジをグラフト化，重層コーティングして厚く付着させた。コラーゲンスポンジは医療用のブタ皮膚由来のコラーゲンを用いた。

頸部気管については，イヌの頸部気管を切除した後に人工気管を移植し，最長5年の観察で，気管の上皮再生は

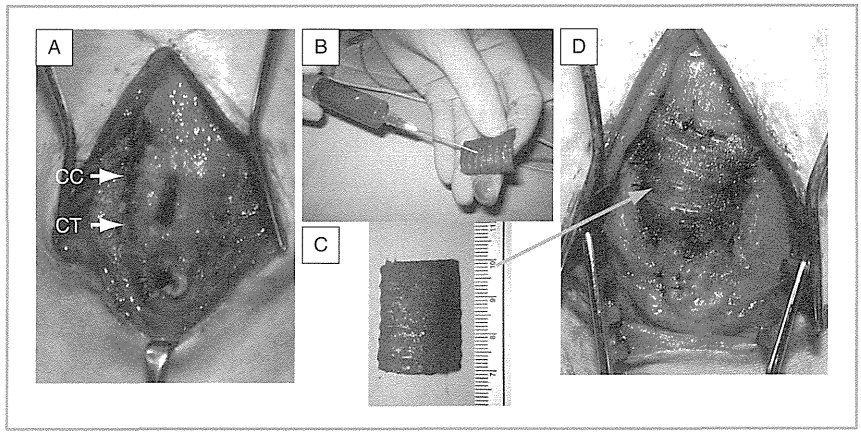

図2　人工気管を用いた再建手術（声門下・頸部気管狭窄例）
A：輪状軟骨（Cricoid Cartilage ； CC）・頸部気管（Cervical Trachea ； CT）の欠損部
B：人工気管を半周分にトリミングし血液を湿潤
C：移植前の人工気管
D：前壁を被覆するように人工気管を縫合固定　（カラーグラビアp4　写真6参照）

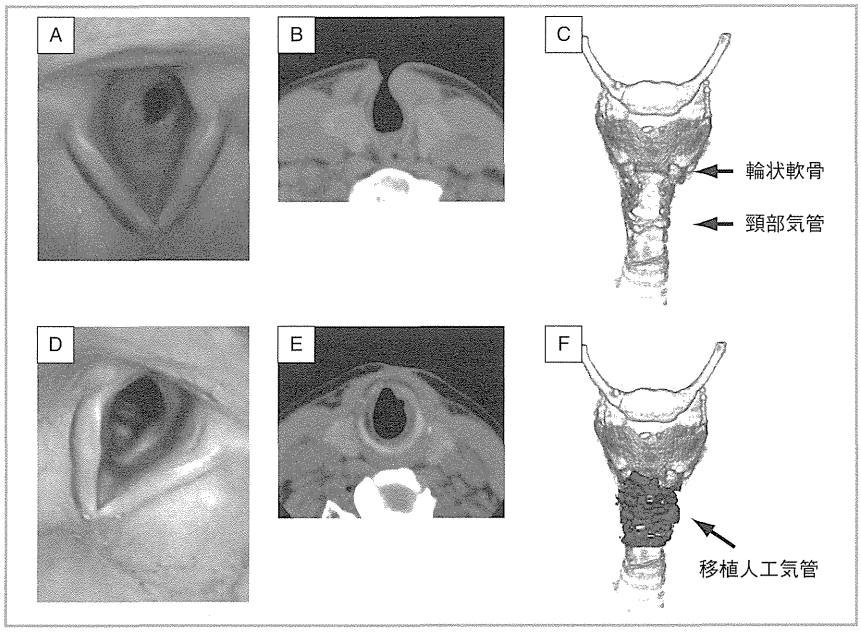

図3　術前術後の画像所見
A：初診時の内視鏡像：声門下の高度狭窄を認め呼吸困難がある。
B：瘢痕切除後，再建術前のCT：気道の内腔は狭小化している。
C：瘢痕切除後，再建術前の3D-CT：輪状軟骨・頸部気管の前壁に欠損を認める。
D：再建術後4年の内視鏡像：内腔面は粘膜に覆われ再狭窄を認めていない。
E：再建術後のCT：気道の内腔は十分保たれている。
F：再建術後の3D-CT：移植人工気管により欠損していた前壁が再建された。
（カラーグラビアp4　写真7参照）

表3　人工気管の実用化を目指した医療機器開発推進研究

・生産ライン構築（GMP/QMS準拠）
　医療機器製造クリーンルーム
　製造手順書作成
　製造・品質・衛生管理

・生物学的安全性試験（GLP準拠）

・臨床試験準備
　臨床試験実施手順書の作成

良好で問題なく経過した。組織学的評価では，炎症所見を認めず，内腔面は再生線毛上皮で覆われていた。再生気管の強度は機械的圧縮試験で正常気管と同程度であった。正常気管との接合部も安定した組織移行がみられ，長期に安全に使用できることがわかった[6)7)]。輪状軟骨については，輪状軟骨の弓部を切除した後に人工気管をトリミングして移植し，最長1年の観察で，一部メッシュが露出したが上皮再生は良好で問題なく経過した[8)]。なお，動物の愛護および管理に関する法律および所属施設の動物実験委員会の指針に従って実験を行った。

臨床症例

大型動物への移植実験で最長5年の経過観察で良好な内腔上皮再生が得られ安全性が確認されたことから，施設内倫理委員会の承認のうえ，2002年より筆者らは，生体内組織再生誘導型の人工気管を用いた気道の再生医療を世界に先駆けて行っている。甲状腺癌の気管浸潤例に対する気管再建と喉頭気管狭窄例に対する病変切除後の二期的再建を行い，現在まで経験した成人11例において最長7年の経過観察で内腔上皮再生が得られている[9)-11)]。声門下・頸部気管狭窄例の気道再建手術，内視鏡およびCT所見を図2，図3に示す。

生体内組織再生誘導型の人工気管は，少数の臨床例における最長7年の観察で，頸部気管と輪状軟骨においては安全性，有効性は確認されている。人工気管はトリミングが可能で，欠損部に適した気道再建手術を容易に安定して実施できるとともに，ほかの部位からの組織採取が不要で手術侵襲を軽減させ，患者のQOL向上に寄与できる。

人工気管の製造方法は完成しており，足場のみを用いるため細胞移植に比べると製造管理や品質管理などのハードルは低い。人工気管が一般医療として広く用いられることが期待されており，現在厚生労働省科学研究費の助成を受け，医療機器としての実用化に向けて，製造環境の整備，製造工程の手順書作成，品質管理の精度向上などGMP/QMS準拠の生産ライン構築に取り組んでいる（表3）。今後，製造から販売を取り扱う企業が決まれば，医療機器として実現するものと期待される。

気管の再生医療研究の動向

2002年，Langerらのグループはヒツジの気管再生を試みたが術後の気道閉塞などの問題があり，臨床応用には至っていない[12)]。2008年にはMacchiariniらによりallograftを用いた気管再建が報告され，死体気管の軟骨構造に患者由来の上皮細胞と軟骨細胞を付加して移植したところ，術後4カ月で内腔を保持しており[13)]，2011年には骨髄単核球と人工気管を移植し術後5カ月で内腔を保持していたと報告した[14)]。

筆者らは気管再生の基礎研究として，上皮化を加速するための線維芽細胞や脂肪組織由来幹細胞の付加技術[15)]，ゲル薄膜を用いた人工材料[16)]，声帯隆起の再生に取り組んでいる。将来的には小児例へ適用可能な吸収性材料からなる新規人工気管の開発が望まれる。

おわりに

生体内組織再生誘導型の人工気管は，動物実験で最長5年の観察，その後の少数の臨床例における最長7年の観察で，頸部気管と輪状軟骨については安全性，有効性は確認されている。製造方法は完成しており，足場のみを用いるため細胞移植に比べると製造管理や品質管理などのハードルは低い。現在GMP/QMS準拠の生産ラインを整備しつつあり，製造から販売を取り扱う企業とのマッチングが得られれば医療機器としての実用化が実現し，これにより患者のQOL向上に寄与できるものと思われる。

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Manufacture of a weakly denatured collagen fiber scaffold with excellent biocompatibility and space maintenance ability

A Nakada[1], K Shigeno[1], T Sato[2], T Kobayashi[1], M Wakatsuki[1], M Uji[1] and T Nakamura[1,3]

[1] Department of Bioartificial Organs, Institute for Frontier Medical Sciences, Kyoto University, Japan
[2] Department of Thoracic Surgery, Kyoto University, Japan

E-mail: nakada@frontier.kyoto-u.ac.jp

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Abstract
Although collagen scaffolds have been used for regenerative medicine, they have insufficient mechanical strength. We made a weakly denatured collagen fiber scaffold from a collagen fiber suspension (physiological pH 7.4) through a process of freeze drying and denaturation with heat under low pressure (1×10^{-1} Pa). Heat treatment formed cross-links between the collagen fibers, providing the scaffold with sufficient mechanical strength to maintain the space for tissue regeneration *in vivo*. The scaffold was embedded under the back skin of a rat, and biocompatibility and space maintenance ability were examined after 2 weeks. These were evaluated by using the ratio of foreign body giant cells and thickness of the residual scaffold. A weakly denatured collagen fiber scaffold with moderate biocompatibility and space maintenance ability was made by freezing at −10 °C, followed by denaturation at 140 °C for 6 h. In addition, the direction of the collagen fibers in the scaffold was adjusted by cooling the suspension only from the bottom of the container. This process increased the ratio of cells that infiltrated into the scaffold. A weakly denatured collagen fiber scaffold thus made can be used for tissue regeneration or delivery of cells or proteins to a target site.

(Some figures may appear in colour only in the online journal)

1. Introduction

Various materials have been developed as 'scaffolds' for regenerative medicine. A typical scaffold of synthetic polymers may contain poly-glycolic acid (PGA), poly-L-lactic acid (PLLA) and poly-lactic-co-glycolic acid (PLGA), and scaffolds of natural polymers contain collagen and gelatin. These scaffolds have been used in clinical practice and yield adequate results [1–7]. However, PGA, PLLA and PLGA scaffolds may cause needless adhesion or toxicity because they may reduce the pH of the surrounding tissue [8–10]. We found that a collagen fiber scaffold manufactured at physiological pH was biocompatible and yielded no adhesion. However, it dissolved before tissue regeneration was complete. Various modifications may therefore be needed [11–14]. Glutaraldehyde (GA) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) are well-known chemical cross-linkers. Although defects may make chemical cross-linkers cytotoxic [15], EDC has a relatively low cytotoxicity and is useful as a cross-linker [12, 14, 16]. UV is used for physical cross-linking; it does not involve toxic chemicals [15], but cannot provide sufficient stiffness in a collagen scaffold [16]. We examined thermal processing as a simple physical cross-linking method. We made a collagen

[3] Author to whom any correspondence should be addressed.

fiber scaffold by freeze drying a collagen fiber suspension with a physiological pH of 7.4. This was followed by heating under negative pressure. Heating formed cross-links between the collagen fibers, providing sufficient strength to maintain a space for tissue regeneration against the pressure of surrounding tissue or collagenase [17]. In this study, mechanical strength is defined as the ability to maintain space for tissue regeneration, rather than mere physical strength; this characteristic is important for regenerative medicine. The scaffold was embedded under the back skin of a rat to determine if it had sufficient biocompatibility and space maintenance ability to be used as a scaffold for regenerative medicine.

Collagen forms a molecular structure under acidic conditions, but forms fibers under neutral conditions, and the modes shift reversibly. Furthermore, it can be denatured by exposure to heat (denatured collagen, which contains 0% superhelix structure, is called gelatin). The temperature by which half of the collagen molecules have lost their superhelix structure is called the 'denaturation temperature'. The denaturation temperature in the dehydrated state is around 112 °C [18]. The collagen fiber scaffold evaluated in this study was freeze dried and heated under negative pressure. Thermal processing above the denaturation temperature introduced various chemical changes including cross-linking [19, 20] and provided mechanical strength [21, 22]. However, as denaturation and mechanical strength of the scaffold increases, the host's foreign-body reaction also increases. As a result, the scaffold is no longer suitable for use in the body. Therefore, it is important to create a scaffold with moderate mechanical strength to minimize foreign-body reaction. In addition, collagen fiber becomes a barrier with increased mechanical strength, and the number of cells invading the scaffold decreases (figure 1(*a*)). Therefore, our scaffold was designed to promote cell infiltration by orienting the collagen fibers (figure 1(*b*)). As an icy crystal is formed perpendicular to a cooling surface, orientation was achieved by cooling a collagen fiber suspension only from the bottom of the container.

To determine the optimal denaturing conditions to create an ideal scaffold with excellent space maintenance ability and biocompatibility, various types of denatured collagen fiber scaffolds with orientation were embedded under the back skin of rats. The optimal observation period was determined to be 2 weeks because it was important to maintain the scaffold at least during the acute or subacute phase of trauma for tissue regeneration. The *in vivo* behavior of these implants was evaluated by determining their space maintenance and biocompatibility scores.

2. Materials and methods

2.1. Manufacturing of a denatured collagen fiber scaffold

The collagen used in this study was atelocollagen extracted from young porcine skin by enzymatic treatment with pepsin (NMP collagen PS, provided by Nippon Meatpackers, Ibaraki, Japan) and consisting of type I (70–80%) and type III collagen.

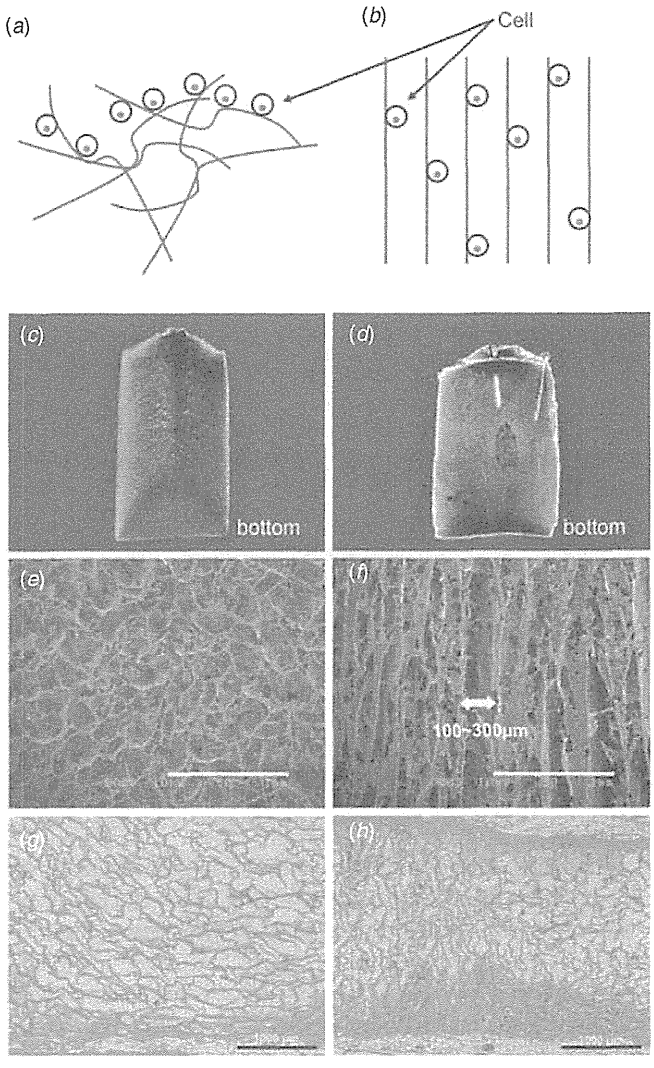

Figure 1. Difference between scaffolds with and without collagen fiber orientation, (*a*) schematic illustration of the collagen fiber without orientation, (*b*) schematic illustration of the collagen fiber with orientation (cases a and b demonstrated cell infiltration into the scaffold, parallel to the collagen fiber), (*c*) macroscopic image of a vertical section of the collagen fiber scaffold without orientation, (*d*) macroscopic image of a vertical section of the collagen fiber scaffold with orientation. (The contrast between figures (*c*) and (*d*) is coordinated to show the direction of collagen fiber in the scaffold.) (*e*) Electron-microscopic image of the collagen fiber scaffold without orientation, (*f*) electron-microscopic image of the collagen fiber scaffold with orientation, (*g*) scaffold without orientation of the collagen fiber (H–E image, 1 week after the operation), (*h*) scaffold with orientation of the collagen fiber (H–E image, 1 week after the operation), scale bars represent 1000 μm in *e–h*.

From this, the collagen mass (pH 7) was made according to the protocol provided with the product. Briefly, 30 g NMP collagen PS was added to 5 l distilled water (0.6% w/v) and the mixture was stirred in the refrigerator overnight to make a collagen solution. After adjusting the pH to 7 with 1N NaOH, the solution was centrifuged to collect the deposit, which was freeze dried to yield a collagen mass. Next, the mass was crushed with a grater to yield particles of approximately 2 × 2 × 2 mm^3 and 6 g was added to 200 ml sterilized Milli-Q water. The mixture was stirred in a Hybrid Mixer (HM-500,

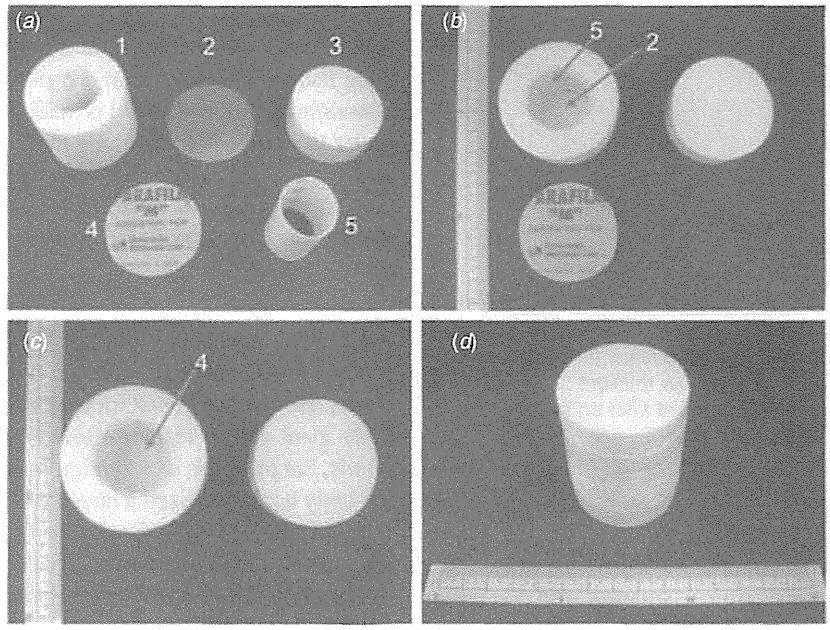

Figure 2. Containers that provide collagen fiber orientation, (*a*) 1. The outer frame is made of styrene foam; 2. Aluminum board; 3. Lid made of styrene foam; 4. Parafilm; 5. The inner frame is a measuring cup made of paper. (*b*) An aluminum board is attached to the bottom of the outer frame with vinyl tape, and the inner frame is installed. (*c*) The volume of the collagen fiber suspension expands as it freezes and may touch a lid made of styrene foam. Parafilm is used to prevent mixing of the styrene foam with the collagen. (*d*) The suspension is covered with a lid made of styrene foam and fixed with vinyl tape. Cooling is applied from the lower side of the container, thus providing orientation to the collagen fiber scaffold.

Keyence, Osaka, Japan) for 2 min, then cooled at 4 °C for more than 30 min. This process was repeated 5 times to yield a 3% w/v uniform collagen fiber suspension. The suspension was stored at 4 °C for 12 h to create a uniform suspension. The pH of the collagen fiber suspension was adjusted to 7.4 with 1N NaOH and stirred in an Ace Homogenizer (HM-500, Nissei, Tokyo, Japan) at 5000 rpm for 30 min. This allowed the internal structure of the collagen fiber scaffold to become uniform. The suspension, which became paste-like, was poured into a container (figure 2) and frozen at −10 °C. Since an icy crystal forms perpendicular to a cooling surface, orientation can be achieved by cooling the collagen fiber suspension only from the bottom of the container (the fibers will orient perpendicular to the bottom). After 12 h, the frozen suspension was freeze dried for 3 days and was heat denatured under low pressure (1×10^{-1} Pa) to form a denatured collagen fiber scaffold. Manufacturing conditions are shown in table 1. Since the pore size of the collagen fiber scaffold within about 5 mm from the cooling surface is too small and the ratio of cell infiltration is small, only that portion of the scaffold >5 mm from the bottom was used. To investigate the internal structure of the scaffold, scanning electron microscopy was performed prior to placement in the experimental animals. After being cut into 1 cm × 1 cm × 5 mm sections (figure 3(*a*)), the collagen fiber scaffold was sterilized with ethylene oxide gas prior to implantation.

Table 1. Manufacturing conditions for the denatured collagen fiber scaffold.

pH	P-temp (°C)[a]	P-time (hours)[b]
7.4	50	6
7.4	100	6
7.4	100	24
7.4	110	6
7.4	120	6
7.4	120	12
7.4	120	18
7.4	120	24
7.4	130	6
7.4	140	0 (no denaturation)
7.4	140	6
7.4	140	9
7.4	140	12
7.4	140	24
7.4	170	5
7.4	170	6

[a] P-temp, processing temperature.
[b] P-time, processing time.

2.2. *Animal and surgical procedures*

Male Wistar rats (500 g, Shimizu Laboratory Supply, Kyoto, Japan) were used for this study. Under anesthesia induced by intraperitoneal injection of sodium pentobarbital (3 mg/100 g body weight), each rat was placed in a prone position. Next, six incisions, measuring about 15 mm in length, were made on the back skin of each rat, and pockets to embed collagen fiber scaffolds were made using Pean forceps. Then the collagen fiber scaffolds were inserted into the pockets. Three pockets on three individual rats were used for each scaffold. That is, three scaffolds were used for each manufacturing condition. After embedding the scaffolds, the skin was closed with

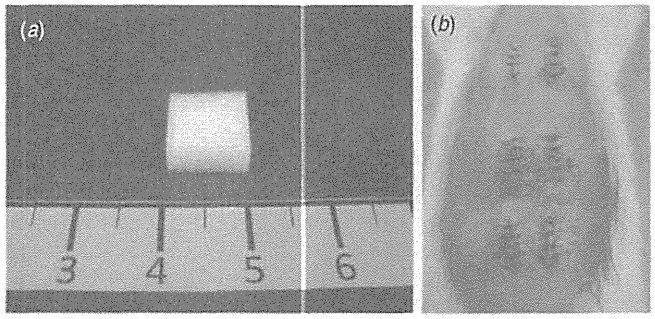

Figure 3. Collagen fiber scaffold and appearance just after it is embedded under the skin, (*a*) collagen fiber scaffold 1 cm × 1 cm × 5 mm, (*b*) appearance just after embedding the collagen fiber scaffold under the back skin of a rat.

3–0 nylon sutures (figure 3(*b*)). Tissue evaluation was performed 2 weeks after the procedure.

In order to evaluate the effect of collagen fiber orientation in the scaffold, three samples with orientation, and 3 samples without orientation were used. In the preliminary study, we found that scaffolds processed at 140 °C for 24 h had poor biocompatibility; therefore, we used these conditions in order to evaluate only the effect of collagen fiber orientation. Tissue evaluation was performed 1 week after the operation, since it is ideal for cellular infiltration of the scaffold to occur in the early stage of implantation.

All surgical experiments were performed according to the Principle of Laboratory Animal Care advocated by the Animal Research Committee of Kyoto University (2007).

2.3. Evaluation

2.3.1. Histology. Two weeks after scaffold implantation, the rats were killed by an overdose injection (intraperitoneal) of sodium pentobarbital. The back skin of each rat was stripped and fixed with 4% paraformaldehyde for more than 72 h at room temperature. All samples were extracted with skin, then dehydrated through ascending alcohol concentrations, embedded in paraffin, serially sectioned (4 μm) in the perpendicular plane, dewaxed and stained with hematoxylin-eosin (H–E). Thereafter, the sections were examined by light microscopy (BIOREVO BZ-9000, Keyence, Osaka, Japan).

2.3.2. Space maintenance ability. Although the scaffold should ideally disappear from the body after a certain period, it must remain *in vivo* at least until tissue regeneration is complete. In this study, we observed a tendency for the thickness of the specimen on the prepared slide to increase as denaturation advanced; thus, the space maintenance ability was scored by using thickness as an index:

Thickness score: TS

(1) The average thickness of three samples = 0 mm. → TS = 1
(2) The average thickness of three samples > 0 mm. → TS = 2 + (Average thickness/1000)

Since no space is identified when TS is below 2, a TS-2 is considered the lowest level required for the scaffold.

Tissue shrinks during the process of preparing a slide. In other words, the thickness of the specimen on the prepared slide becomes thinner than 5 mm even if the prepared slide containing a 5 mm scaffold is made just after being embedded under the skin. Therefore, the thickness of the collagen fiber scaffold 2 weeks after the operation is actually thicker than that on the prepared slide. In this study, space maintenance ability was evaluated based on the thickness of the specimen on a prepared slide.

2.3.3. Biocompatibility. Once the living body recognizes a foreign body, macrophages are dispatched to eliminate the object. However, the macrophages fuse and become a foreign body giant cell in response to a large foreign body. In other words, the presence of a foreign body giant cell indicates that the body has recognized a large foreign body. In this study, the scaffold in which many foreign body giant cells were identified caused a strong inflammatory reaction and could never be used to construct tissue, even if it remained in place 2 weeks after surgery. Therefore, 'the degree of foreign body' of the scaffold is evaluated by the following score.

Biocompatibility score: BS

(1) BS-1: foreign body giant cells are identified around the embedded collagen fiber scaffold.
(2) BS-2: foreign body giant cells are identified around only a portion of the embedded collagen fiber scaffold.
(3) BS-3: a few foreign body giant cells are identified around the embedded collagen fiber scaffold. (Since a foreign-body reaction appeared below BS-3, this is considered the lowest level of biocompatibility.)
(4) BS-4: no foreign body giant cells are identified around the embedded collagen fiber scaffold.

3. Results

Macroscopic and electron microscopic images of the collagen fiber scaffolds were collected. There was no orientation of collagen fibers unless the suspension was cooled from one direction (figures 1(*c*), (*e*)). Cooling from the bottom of the container yielded collagen fibers arranged perpendicular to the cooling surface, and the pore sizes of this scaffold measured 100–300 μm (figures 1(*d*), (*f*)). The infiltration of cells perfusing the scaffold slowed when the collagen fiber was not oriented properly (figure 1(*g*)). This result was the same for all samples processed at 140 °C for 24 h without collagen fiber orientation. On the other hand, infiltration improved when the collagen fiber in the scaffold was oriented in a single direction (figure 1(*h*)). This result was the same for all samples processed at 140 °C for 24 h with collagen fiber orientation.

We evaluated the relationship between manufacturing conditions and the properties of the created collagen fiber scaffold (table 2). Denaturation of the collagen at 140 °C for 6 h produced a collagen fiber scaffold with TS greater than 2 and BS-3 at 2 weeks after implantation. However, denaturation at 140 °C for more than 12 h yielded no biocompatibility (BS-1). 'Impossible to evaluate' indicates that it was impossible to evaluate the collagen fiber scaffold because it

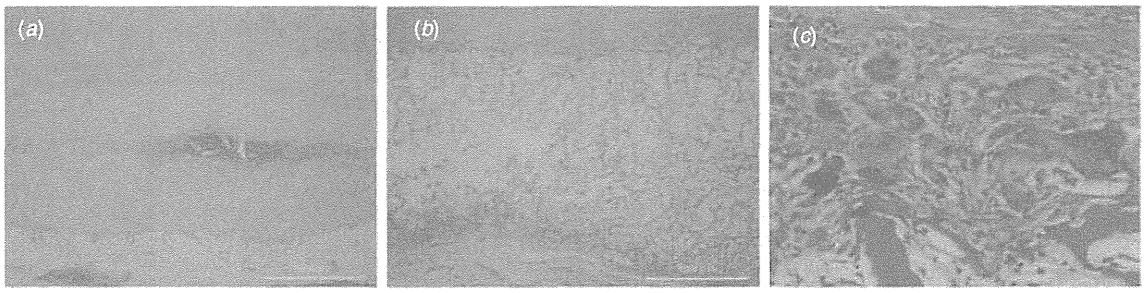

Figure 4. Collagen fiber scaffold with and without thermal denaturation at 140 °C for 24 h, (*a*) collagen fiber scaffold without thermal denaturation after 1 week, (*b*) collagen fiber scaffold with thermal denaturation (140 °C for 24 h) after 2 weeks, (*c*) the foreign body giant cell identified around the scaffold (figure 4(*b*)), the scale bars represent 1000 μm in *a*, *b* and 50 μm in *c*. (H–E image.)

Table 2. Relationship between manufacturing conditions and the properties of the denatured collagen fiber scaffold.

pH	P-temp (°C)[a]	P-time (hours)[b]	Thickness (μm)	Av (μm)[c]	TS[d]	BS[e]
7.4	50	6	0, 0, 0	0	1	IE (3)[f]
7.4	100	6	0, 0, 0	0	1	IE (3)
7.4	100	24	0, 0, 0	0	1	IE (3)
7.4	110	6	0, 0, 0	0	1	IE (3)
7.4	120	6	0, 0, 0	0	1	IE (3)
7.4	120	12	0, 0, 0	0	1	IE (3)
7.4	120	18	0, 0, 0	0	1	IE (3)
7.4	120	24	275, 334, 833	481	2.48	1
7.4	130	6	0, 0, 0	0	1	IE (3)
7.4	140	0	0, 0, 0	0	1	IE (4)
7.4	140	6	701, 827, 1340	956	2.96	3
7.4	140	9	1263, 1571, 1692	1509	3.51	2
7.4	140	12	1215, 1286, 1930	1477	3.48	1
7.4	140	24	1810, 2025, 2286	2040	4.04	1
7.4	170	5	1619, 1691, 2000	1770	3.77	1
7.4	170	6	1691, 2000, 2029	1907	3.91	1

[a] P-temp, processing temperature.
[b] P-time, processing time.
[c] Av, average thickness of 3 samples.
[d] TS, thickness score.
[e] BS, biocompatibility score.
[f] IE, impossible to evaluate.

had disappeared 2 weeks after it was embedded. This indicated insufficient denaturation. The comment noted in the table as 'Impossible to evaluate' was therefore considered to indicate BS-3. However, as for 140 °C for 0 h, it was evaluated as BS-4.

4. Discussion

A collagen microfibril contains five collagen molecules [23–26]; collagen fibrils are formed by aggregation of these microfibrils. Collagen fiber is an assembly of these fibrils. The main epitope of a collagen fiber is in the nonspiral structural telopeptides at both ends of the collagen molecule, and the remainder of the molecule differs little between animal classes.

The collagen used in this experiment is atelocollagen, which is produced by the elimination of the antigenic telopeptides by pepsin. The biocompatibility of atelocollagen without denaturation is very high because BS is 4 at 1 week after embedding. However, there is poor infiltration of cells because spaces for tissue regeneration are not maintained (figure 4(*a*)). Moreover, it was nearly dissolved 2 weeks after embedding. On the other hand, although the mechanical strength increased and sufficient space was maintained even 2 weeks after the operation, antigenicity of the collagen fiber scaffold increased and tissue construction could not be identified when thermal processing was performed at 140 °C for 24 h. Therefore, the collagen fiber scaffold was recognized as a foreign body with low biocompatibility (figure 4(*b*)). This resulted in a foreign body giant cell with multiple nuclei surrounding the scaffold (figure 4(*c*)). Thus, mechanical strength was inversely proportional to biocompatibility. It is thought that optimal 'weak denaturation' occurs at a processing temperature of 140 °C between 0 and 24 h; we concluded the optimal time was 6 h (figure 5(*a*)). Figure 5(*a*) is drawn based on the results of three samples at 140 °C for 0, 6, 9, 12 and 24 h (shown in table 2). Figure 5(*a*) illustrates that although biocompatibility is good, the space maintenance ability is inferior when processing time is shorter than 6 h. On the other hand, the space maintenance ability is superior, but biocompatibility declines when the processing time is longer than 6 h. Since collagen denaturation progresses over time [27], the BS curve shown in figure 5(*a*) declined even if the BS score was the same. The graph accurately reflected the properties of the collagen fiber scaffold indicated in table 2.

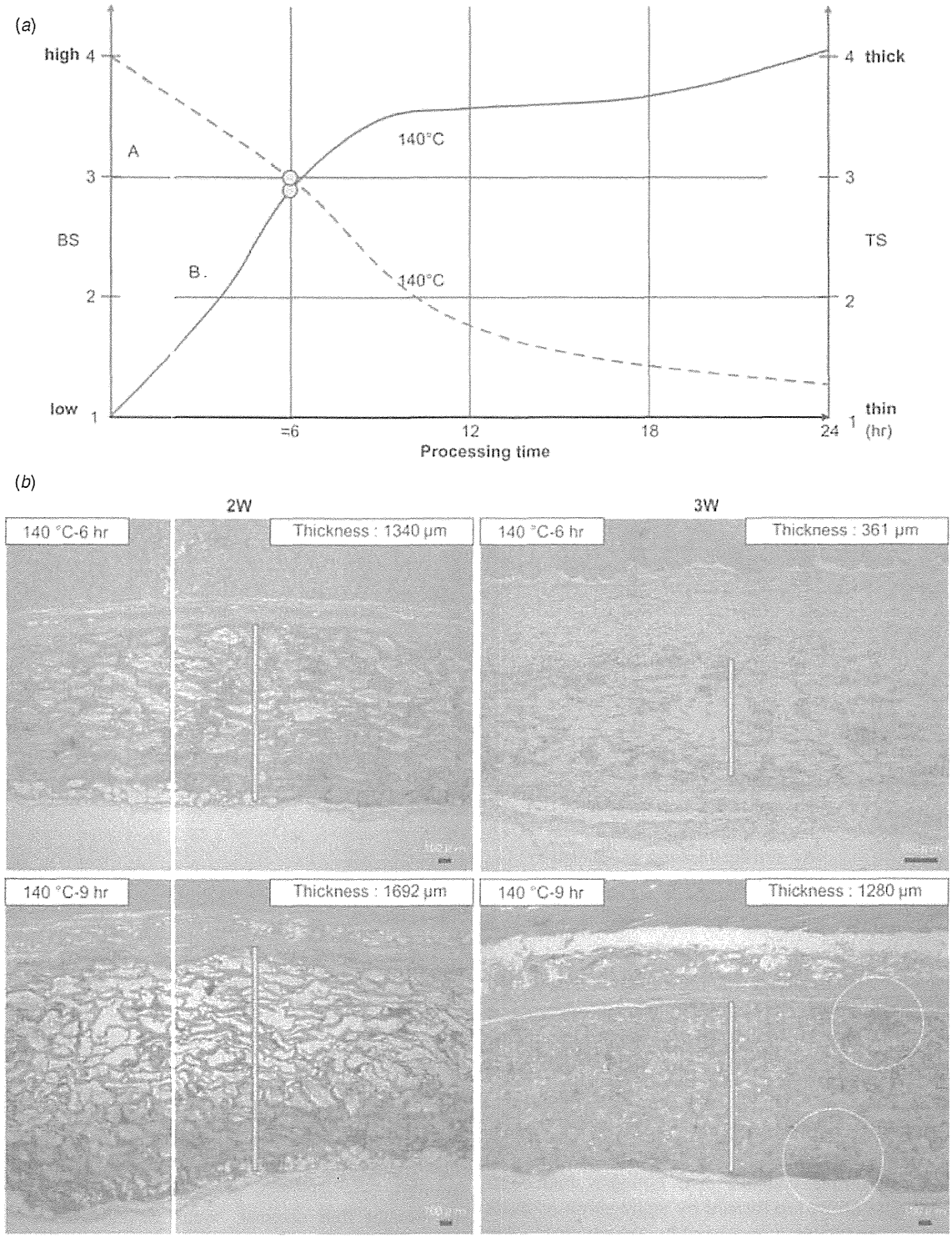

Figure 5. Relationship between thermal denaturation processing time and the space maintenance ability and biocompatibility of a collagen fiber scaffold: (*a*) the red dashed line represents biocompatibility, and the blue line represents space maintenance ability. Line A represents the lowest level of biocompatibility (BS-3) and line B represents the lowest level of space maintenance ability (TS-2) required for the scaffold. (*b*) Thermal denaturation conditions are indicated on the left of each figure and scaffold thickness is indicated on the right. The yellow vertical line in each figure indicates the thickness of each scaffold. The part yellow circled indicates sites of inflammatory cell infiltration. The scale bars represent 100 μm. (H–E image.)

Cells infiltrate the collagen fiber scaffold along collagen fibers; thus, the infiltration rate improved when the scaffold with collagen fiber orientation was used. This is likely because continuous holes are made in the scaffold. This improvement is also likely to ease neovascularity. It is important to ensure blood flow around the region of tissue regeneration to maintain the infiltrated cells. Moreover, when a collagen fiber scaffold containing disseminated cells is implanted, neovascularity in

the early phase is also required to maintain the viability of the disseminated cells. Therefore, orientation of the collagen fibers in a scaffold should improve tissue regeneration. Particularly in the case of hard biomaterials used for bone regeneration, continuous holes greatly improve cell infiltration [28, 29]. Bone tissue engineering reports have suggested the utility of a complex of collagen and glycosaminoglycan (GAG). Tierney *et al* reported that a scaffold with suitable mechanical and biological properties was produced by treatment at 150 °C for 48 h [30] and Haugh *et al* reported scaffolds treated at 150 °C for 120 h or 180 °C for 24 h [31]. The results of both thermal processing methods are far stronger than our results. However, we also found that strong thermal processing at 140 °C for 24 h is suitable for producing a collagen scaffold with the desired mechanical properties. Drexler *et al* used the dehydrothermal cross-linking method and reported that thermal processing at 140°C for 24 h improved mechanical properties and biostability [17]. However, these reports utilized collagen molecules under acidic conditions. Since the properties of collagen molecules are different from those of the collagen fibers used in this study, it is difficult to compare the thermal processing conditions mentioned by Drexler *et al* with the thermal processing condition used in this study (140 °C for 6 h).

Pore size can be controlled by freezing temperature; it becomes smaller as the freezing temperature decreases. Although we made a weakly denatured collagen fiber scaffold by freezing at −80 °C [32, 33], the pore sizes were small and few cells infiltrated the scaffold. Since the pore sizes of the present scaffold measured from 100 to 300 μm, freezing at −10 °C is considered suitable for cell infiltration.

Although the results of this study demonstrated that the most suitable denaturation condition is 140 °C for 6 h, it is possible to apply 140 °C for 5 h for tissue regeneration, as shown in figure 5(*a*). Under these conditions, biocompatibility is slightly improved, while space maintenance ability decreases.

When the scaffolds manufactured under 140 °C for 6 h and 140 °C for 9 h conditions were evaluated at 2 and 3 weeks after the operation, the longer treated scaffold had a greater thickness, while the more briefly heated scaffold demonstrated better biocompatibility, a difference that increased by 3 weeks. Inflammatory cell infiltration was identified in scaffolds processed at 140 °C for 9 h as soon as 3 weeks after operation (figure 5(*b*)). This is consistent with the trend demonstrated in figure 5(*a*).

The results of denaturation at 100 °C and 120 °C are shown in figure 6. The TS at 100 °C for 6 h and 24 h are 1 and the BS at 100 °C for 6 h and 24 h are 3. Since 100 °C is below the denaturation temperature, the line indicating BS is considered almost parallel to the horizontal axis. Denaturation at 120 °C for 18 to less than 24 h (time x) also yielded a scaffold with space maintenance ability and biocompatibility. Therefore, other manufacturing conditions may produce an adequate scaffold. In a previous study, we noted that although the scaffold processed at 120 °C for 24 h was always present even 2 weeks after the operation, the scaffold processed at 120 °C for 23 h was not present in about 50% of cases

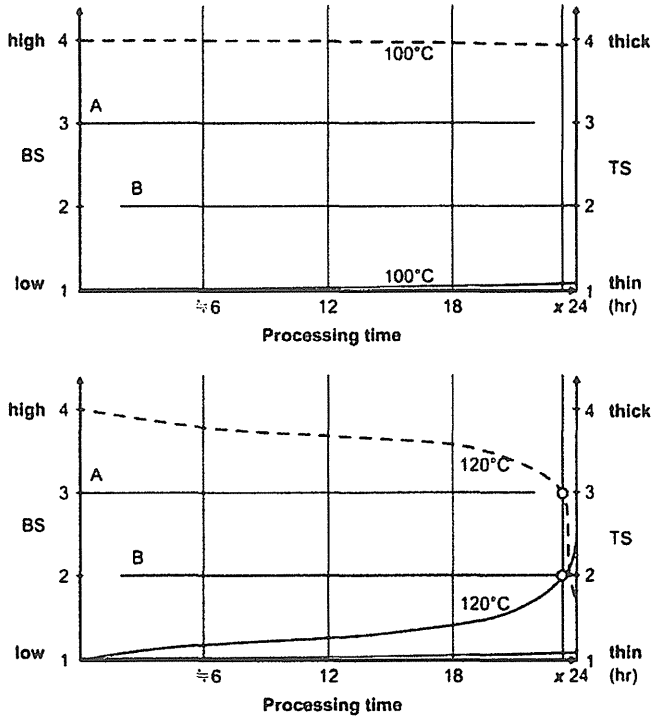

Figure 6. Relationship between thermal denaturation times and the space maintenance ability and biocompatibility of the collagen fiber scaffold. The red dashed line represents biocompatibility, and the blue line represents space maintenance ability. Line A represents the lowest level of biocompatibility (BS-3) and line B represents the lowest level of space maintenance ability (TS-2) required for the scaffold. Time, x, indicates processing time when the denaturation temperature was 120 °C, suggesting that the scaffold is similar to that made at 140 °C for 6 h.

($n = 6$). We believe that the scaffold should remain in place for at least 2 weeks in order to effectively function as a scaffold, so a TS at 120 °C for 23 h is considered to be '1'. Therefore, the time, x, should be between 23 h and 24 h.

A similar experiment is underway using a 6% weakly denatured collagen fiber scaffold. Although the BS was 3, like that of the 3% weakly denatured collagen fiber scaffold 2 weeks after embedding, cells infiltration was poor. This was because the high viscosity of the 6% collagen fiber suspension made it difficult to orient the collagen fibers. In addition, partition of the denatured collagen fiber is thick. A 6% collagen scaffold with orientation can be made occasionally. In such cases, cells infiltrate even to the center of the scaffold along the collagen fiber 2 weeks after the operation. However, few cells' infiltration can be identified in scaffolds without orientation. Therefore, orientation becomes more important when the viscosity is high.

Future experiments will investigate the optimal scaffold strength for specific organs, while reducing the density of the collagen fiber suspension to 6% or less. The shape of our scaffold remains smooth if it is pressed on a plate under conditions at which water is being fully absorbed. Although almost all collagen scaffold materials are made from collagen molecules under acidic conditions, this property is not apparent in acidic collagen scaffolds, including the ones examined in our previous study. The recovery of the scaffold's smooth surface

may contribute to space maintenance for tissue regeneration *in vivo*. Moreover, our scaffold is easily infiltrated with cells if it is pressed several times in cell suspension. This property is useful in the field of cell transplantation [34]. Recently, research efforts to develop a tissue with a three-dimensional structure *in vitro* have been rapidly advancing, and the collagen scaffold developed in this study will be a useful material in various fields.

5. Conclusions

We concluded that collagen denaturation at 140 °C for 6 h produced a scaffold for tissue regeneration with superior space maintenance ability and biocompatibility. Moreover, we suggest that orientation of the collagen fiber in the scaffold facilitates cell infiltration. The concentration of collagen fiber suspension was 3% w/v in this study, but it is possible to control the mechanical strength or the period the scaffold remains *in vivo* by changing the concentration. However, further studies will be necessary to elucidate these conditions. By repeating this process, we think the most suitable scaffold can be produced for any given purpose.

Acknowledgments

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Clinical Trials

Research Article Open Access

Current Environment for Clinical Research with Medical Devices in Hospitals in Japan

Shota Hamada[1], Yukie Yamauchi[1], Osamu Miyake[1], Motoko Nakayama[1], Haruko Yamamoto[2] and Koji Kawakami[1]*

[1]*Department of Pharmacoepidemiology, Graduate School of Medicine and Public Health, Kyoto University, Kyoto, Japan*
[2]*Department of Advanced Medical Technology Development, National Cerebral and Cardiovascular Center, Osaka, Japan*

Abstract

Background: Medical devices are continuously being improved in routine clinical practice. As necessary, new or additional clinical data for an investigational medical device is collected through clinical research and/or registered clinical investigations. We conducted a questionnaire survey to determine the current environment for clinical research with medical devices, particularly focusing on infrastructure and human resources in hospitals.

Methods: The questionnaire for this study included 6 main topics: experience of clinical research, in-hospital manuals, issues on clinical research, related regulations, and effectiveness of a guidance published by the Medical Engineering Technology Industrial Strategy Consortium. The questionnaire was mailed to all 10 core clinical research centers and 30 major clinical trial institutions at the time of survey in Japan.

Results: Eighteen hospitals (45%) provided responses. Relatively few clinical research activities with medical devices had been conducted in each hospital, and two-thirds of respondents thought low number of clinical research activities was problematic. A shortage of experts in medical devices was also raised as an important challenge. Most of the hospitals had established in-hospital manuals for clinical research with medical devices; however, specific features required for the evaluation of medical devices might not be included in the manuals. Many hospitals had too few clinical research coordinators (CRCs) for support of clinical research with medical devices, but half of the hospitals could not afford to increase the number of CRCs.

Conclusion: Our study revealed that the current environment for clinical research with medical devices in hospitals has been partly organized, but it was suggested that a shortage of experts, the complexity of the regulatory system, and a need for financial support are remaining issues.

Keywords: Medical device; Clinical research; Clinical trial; Questionnaire survey; Clinical research coordinator

Introduction

Medical devices play key roles in diagnosis and treatment of diseases in modern healthcare. Unlike drugs, medical devices are continuously improved in routine clinical practice during the development and post-marketing phases to meet the needs of medical staff and patients. However, not all of advanced medical devices used in other countries are available in Japan [1]. Correction of this problem requires establishment of regulations related to development of medical devices and development or improvement of human resources, infrastructure and funding for clinical research and registered clinical investigations with medical devices. In this paper, clinical research is defined in a limited sense as research activities not including registered clinical investigations with Good Clinical Practice (GCP) for a marketing approval application.

During the development process, investigational medical devices are firstly evaluated based on clinical evidence including clinical data such as literature data and/or clinical experience. In response to the needs of new or additional clinical data, clinical research and/or registered clinical investigations are conducted. In particular, an innovative and/or invasive medical device for which clinical data are required for a marketing approval application under the Pharmaceutical Affairs Law (PAL) is evaluated in registered clinical investigations in accordance with the Ministry of Health, Labour and Welfare (MHLW)'s Ministerial Ordinance on GCP for Medical Device. Such clinical investigations are mostly sponsored by medical device companies. Once the safety and effectiveness of a medical device have been evaluated and ensured by the Pharmaceuticals and Medical Devices Agency, the regulatory authority in Japan, and subsequently approved by the MHLW, the medical device becomes accessible to medical staff and patients across the country. In contrast, clinical research with medical devices are predominantly initiated by clinicians in hospitals and generally conducted under permission of each hospital, in accordance with the Ethical Guideline for Clinical Research [2]. Clinical research assures timely evaluations of prototypes of medical devices with novel or altered technologies and with improved usability and/or performance.

In Japan, clinical research and registered clinical investigations are regulated separately and the system is complicated. Clinical investigations are regulated more clearly than clinical research and must be conducted in accordance with the PAL and GCP. Unapproved medical devices are regulated by the PAL, and therefore there was a concern that the supply of unapproved medical devices for clinical research conducted in hospitals constitutes a breach of the PAL. This background caused problems when companies make decisions on supplying medical devices to be tested in clinical research.

Recently, the MHLW released two notices regarding clinical

***Corresponding author:** Koji Kawakami, Department of Pharmacoepidemiology, Graduate School of Medicine and Public Health, Kyoto University, Yoshidakonoecho, Sakyo-ku, Kyoto, Japan, Tel: +81-75-753-4459; Fax: +81-75-753-4469; E-mail: kawakami.koji.4e@kyoto-u.ac.jp

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research with unapproved medical devices [3,4]. These notices indicated that supply of unapproved medical devices for clinical research is exempted from the PAL. To clarify these notices, the Medical Engineering Technology Industrial Strategy Consortium (METIS) published a document entitled "Guidance on clinical research with unapproved medical devices" [5] to streamline the process of clinical research with medical devices. This guidance includes information on the overall picture of clinical research, overviews of related regulations, classification of medical devices, practical considerations at each stage of clinical research, checklists for protocol and informed consent form, and relevant templates for documents such as a collaborative research agreement.

This guidance further defined the regulatory requirements for clinical research with medical devices that are not regulated by the PAL or GCP; however, the current environment for conducting such clinical research in hospitals remains unclear. In Japan, individual hospitals are relatively small and are scattered nationwide, and this situation presents a barrier to efficient development of medical devices and drugs. Therefore, we conducted a questionnaire survey to determine the current environment for clinical research with medical devices, particularly focusing on infrastructure and human resources in hospitals, and to identify issues related to the conduct of clinical research from a hospital perspective.

Materials and Methods

A questionnaire for this study was developed to examine the current environment for clinical research with medical devices in hospitals. The questionnaire included 6 main topics: experience of clinical research, in-hospital manuals, issues on clinical research, roles and sufficiency of support staff, related regulations, and effectiveness of the METIS guidance. The support staff refers to as Clinical Research Coordinators (CRCs) who support clinical research and/or registered clinical investigations. Most of the questions were multiple-choice for the purpose of reducing the time and effort of the respondents, but free descriptions were also obtained as necessary (Table 1).

The survey was conducted between 23 March and 25 April 2012. The questionnaire was mailed to directors of support offices for clinical investigations at all 10 core Clinical Research Centers (CCRCs) and 30 Major Clinical Trial Institutions (MCTIs) at the time of survey in Japan. The MHLW has designated these hospitals for financial support for human resources and infrastructure for smooth and efficient conduct of clinical research and registered clinical investigations [6]. It is particularly important to understand the current status of clinical research with medical devices in these hospitals since they have key roles in development of medical devices and drugs. Data were compiled using Microsoft Office Excel 2010.

Results

Eighteen hospitals (45%) responded to our questionnaire, but some respondents did not answer all of the questions. The reported experience of clinical research with approved or unapproved medical devices in each hospital are shown in Table 2. Relatively few clinical research activities with medical devices had been conducted in the last 2 years. The median number of clinical research activities with medical devices was 5 per hospital when calculated with experience in 12 hospitals where had reported experience of at least one clinical research activity with medical devices, with considerable variation among the hospitals (range, 1-22 per hospital).

The results from the questions on preparation of in-hospital manuals for clinical research and registered clinical investigations with medical devices are shown in Table 3. Thirteen hospitals had established manuals for clinical research with medical devices, and 3 of these hospitals had manuals for clinical research in compliance with GCP. Manuals for clinical research with medical devices were the same as those used for drugs in 15 hospitals, and only 6 of these hospitals have manuals that cover clinical research with both approved and unapproved medical devices. Similarly, manuals for registered clinical investigations with medical devices were the same as those used for drugs in most hospitals.

There were several general issues on conduct of clinical research with medical devices (Figure 1). In particular, two-thirds of respondents thought that the much lower number of clinical research activities with medical devices compared to those with drugs was problematic. In this context, 4 respondents suggested that there was a shortage of experts in this field and/or indicated a lack of applicability of experience in clinical research with drugs due to methodological differences. There was an opinion that the low number of clinical research activities is one of the reasons why they could not hire staff specialized in medical device. Five respondents felt that separate management of investigational medical devices for clinical research and medical devices for routine practice was complicated. One of respondents' requests for medical device companies is more proactive technical support, for example, assistance on how to manage investigational medical devices.

The roles of CRCs were mainly to support registered clinical investigations in more than a half of the hospitals (Table 4). The median number of CRCs in each hospital was 7 (range, 2-18). Seven hospitals assigned a median of 2 CRCs (range, 1-5) as staff specialized in clinical research and registered clinical investigations with medical devices. Most respondents thought that more CRCs were needed in their hospitals, but half of the hospitals could not afford to increase the number of CRCs. These results suggest a common trend of an insufficient number of CRCs in the hospitals, particularly for support of clinical research with medical devices.

The notification on supply of unapproved medical devices issued by MHLW was highly recognized (16/18, 89%). Out of those who answered that they know the notification, 10 respondents agreed that clinical research will be more activated by the notification. To the question whether regulations should be eased so that unapproved medical devices can be provided to researchers at the request of companies, the respondents were almost equally divided between those who agreed and disagreed. In addition, out of 6 respondents disagreed that clinical research will be more activated by the notification, 4 respondents agreed to the aforementioned question whether regulations should be eased.

The METIS guidance was highly appreciated. The respondents thought the guidance was useful for physicians, dentists, CRCs and administrative officers associated with clinical research, including staff in charge of ethical review. The followings were listed as especially useful among the contents of the guidance: procedures for clinical research with medical devices, a template for a collaborative research agreement, classification of medical devices, and methods for management of investigational medical devices. In particular, the visual summaries shown as flowcharts and tables, including the overall picture of clinical research and the classification of medical devices, were highly praised. Some additions to the current guidance were proposed, including a template for a study protocol and methods for dealing with malfunctions of investigational medical devices.

Topic	Question [Question type]	Response options
Experience of clinical research	Which and how much experience does your hospital have related to clinical research with medical devices, excluding experience related to registered clinical investigation? [Filling in numbers of all applicable experience within the last 2 years]	• Experience with approved medical devices within the approved indications • Experience with approved medical devices under off-label use • Experience with unapproved medical devices • No experience
In-hospital manuals	Have manuals for clinical research been established? [Single answer]	• Yes / No
	If no, are manuals for clinical research in preparation? [Single answer]	• Yes / No
	Are the manuals for clinical research with medical devices the same as those used for drugs? [Single answer]	• Yes / No
	Are the manuals for registered clinical investigations with medical devices the same as those used for drugs? [Single answer]	• Yes / No
Issues on clinical research	Which issue do you face when conducting clinical research with medical devices? [Multiple answers allowed]	• Lack of applicability of experience in clinical research with drugs. • Shortage or none of experts in medical devices in the hospital. • More difficult to recruit participants compared to clinical research with drugs. • Separate management of investigational medical devices for clinical research and medical devices for routine practice is complicated. • Much lower number of clinical research activities with medical devices compared to those with drugs. • Insufficient support from medical device companies.
	If any comments on cooperation from medical device companies, please specify. [Open-ended]	
	If any other issues, please specify. [Open-ended]	
Roles and sufficiency of support staff	What are CRCs in your hospital involved in? [Single answer]	• Only registered clinical investigations • Mainly registered clinical investigations • Both at about the same level
	Do you think the number of CRCs in your hospital is sufficient? [Single answer]	• Yes / No
	Does your hospital plan to increase the number of CRCs? [Single answer]	• Yes / No
Related regulations	Do you agree that clinical research will be more active thanks to the notification "Application of Pharmaceutical Affairs Law to supply of unapproved medical devices used in clinical research" issued on March 2010? [Single answer]	• Agree / Disagree / Do not know the notification
	Do you agree that regulations should be eased so that unapproved medical devices can be provided to researchers at the request of companies? [Single answer]	• Agree / Disagree
Effectiveness of the METIS guidance	What kinds of professional are likely to find the METIS guidance useful? [Multiple answers allowed]	• Medical doctor / Dentist / Nurse / Pharmacist / Other paramedics / CRC / Officer of clinical research center / Member of ethical committee
	If other, please specify. [Open-ended]	
	Which part of the METIS guidance is considered to be useful? [Multiple answers allowed]	• Classification of medical devices • Differences in regulatory systems between non-registered clinical research and registered clinical investigations • Procedures for clinical research with unapproved medical devices • Preparation of protocol and informed consent form • Templates of contracts • Management of investigational medical devices • Protection of participants in clinical research • Interruption, cancellation, and termination of clinical research • Check-lists for protocol and informed consent form
	If other, please specify. [Open-ended]	

Table 1: Contents of Questionnaire.

Discussion

Medical devices are continuously being improved in routine clinical practice. As necessary, new or additional clinical data for an investigational medical device is collected through clinical research and/or registered clinical investigations. To our knowledge, this report is the first survey of the environment for clinical research with medical devices in hospitals in Japan.

In-hospital manuals for clinical research with medical devices were established or in preparation at the time of the study; however, two major issues with these manuals were identified that might affect the quality of clinical research. The first is that some hospitals prepared the manuals in compliance with GCP. Clinical research with medical devices is not necessarily conducted in compliance with GCP under the current regulatory system, and the conduct of clinical research with such high level of quality is an overreach and a waste of time,

	n (%)
With approved medical devices	12 (67%)
Within the approved indications	11 (61%)
Under off-label use	9 (50%)
No reply	5 (28%)
With unapproved medical devices	9 (50%)
None or unknown	5 (28%)
No reply	4 (22%)

Table 2: Experience with clinical research with medical devices in each hospital (N=18).

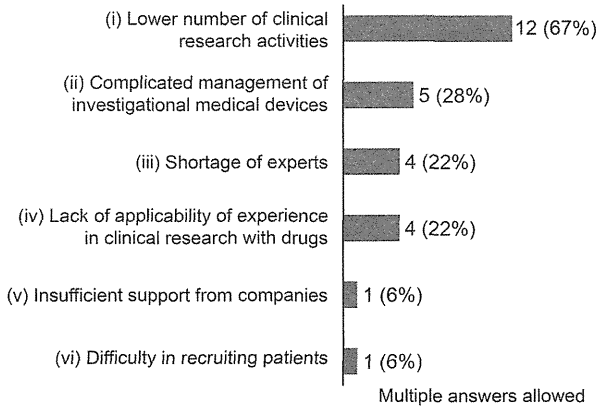

Figure 1: Issues on clinical research with medical devices.
(i) Much lower number of clinical research activities with medical devices compared to those with drugs.
(ii) Separate management of investigational medical devices for clinical research and medical devices for routine practice is complicated.
(iii) Shortage or none of experts in medical devices in the hospital.
(iv) Lack of applicability of experience in clinical research with drugs.
(v) Insufficient support from medical device companies.
(vi) More difficult to recruit participants compared to clinical research with drugs.

	Yes	No	No reply
	n (%)	n (%)	n (%)
Have manuals for clinical research been established?	13 (72%)	4 (22%) [a]	1 (6%)
Are the manuals for clinical research with medical devices the same as those used for drugs?	15 (83%)	1 (6%)	2 (11%)
Are the manuals for registered clinical investigations with medical devices the same as those used for drugs?	13 (72%)	4 (22%)	1 (6%)

a: All the 4 hospitals had not established manuals for clinical research, but had been preparing at the time of the survey

Table 3: Manuals for clinical research and registered clinical investigations with medical devices (N=18).

money and effort of researchers and companies. The complexity of regulatory systems might underlie this problem. Different regulatory systems are applied separately to clinical research and registered clinical investigations [7,8]. A possible solution may be to unify the two systems for one system like investigational device exemption in the United States.

The second issue is that the manuals for clinical research, as well as registered clinical investigations, with medical devices were the same as those used for drugs in most of the hospitals (Table 3). This implies that specific features required for the evaluation of medical devices are not included in the manuals. The respondents indicated substantial differences in procedures in clinical investigations and clinical research with drugs and medical devices, and experience in clinical research with drugs cannot always be applied to medical devices (Figure 1). This issue may arise from insufficient experience with clinical research and clinical investigations with medical devices; thus, specific procedural descriptions might not be included in the in-hospital manuals.

Relatively few clinical research activities with medical devices had been conducted in each hospital. Therefore, the experience and findings from clinical research with medical devices should be shared among hospitals and medical device companies to improve development of medical devices to the extent possible. The METIS guidance will be updated based on the needs of medical staff and medical device companies and on changes in the environment for medical device development. The updated guidance is expected to include some case studies and more specific procedural advice, which should partly complement the knowledge and experience in hospitals and companies.

A shortage of experts in medical devices was raised as an important challenge (Figure 1). In Japan, the delay of clinical research and clinical investigations with drugs and medical devices following basic research is often pointed out [9]. In particular, the characteristics of medical devices vary widely and multidisciplinary knowledge is needed in medical device development. A recent comparison of undergraduate and graduate education at universities in Japan and the United States for development of human resources for promotion of development and application of medical devices led to several proposals [10]. These included continuous funding for the centers of excellence in research and education as necessary, quality control of educational programs, accreditation for educational programs, and strengthening of regulatory science education. Such education can also enhance the effectiveness of on-the-job training and achieve flexible application of knowledge.

There are several limitations that affect the validity of the study. We sent the questionnaire to all CCRCs and MCTIs designated by the MHLW at the time of the survey, but the response rate was only 45% and some respondents did not answer all of the questions. An unbalanced distribution of non-respondents and respondents limits the internal validity of the study. Generalizability of the study may also be limited because CCRCs and MCTIs are highly organized compared to most hospitals in Japan. Further studies need to include smaller hospitals because innovation in medical devices can occur anywhere. In addition to the issues raised by the present study, other challenges may exist in medical device development in Japan, as discussed in the United States [11]. Issues and challenges will vary with changes in the regulatory system and accumulation of experience in medical device development

	n (%)
What are CRCs in your hospital involved in?	
- only registered clinical investigations	2 (11%)
- mainly registered clinical investigations, but also clinical research	11 (61%)
- both registered clinical investigations and clinical research at about the same level	4 (22%)
No reply	1 (6%)
Do you think the number of CRCs in your hospital is sufficient?	
- sufficient	2 (11%)
- insufficient, but plans to increase	8 (44%)
- insufficient, and no plan to increase	7 (39%)
No reply	1 (6%)

Table 4: Roles and sufficiency of clinical research coordinators (CRCs) (N=18).

in hospitals and companies, and these should be identified and resolved on an ongoing basis.

In conclusion, our study revealed that the current environment for clinical research with medical devices in hospitals has been partly organized, but it was suggested that a shortage of experts, the complexity of the regulatory system, and a need for financial support are remaining issues. Measures to meet these challenges should be taken to create a positive cycle of medical device development.

Conflict of Interest

All authors declare no conflict of interest with regard to this work.

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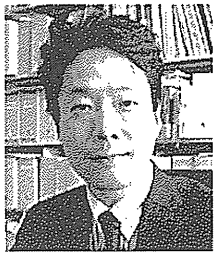
わが国の臨床研究の現状と未来

Clinical research in Japan——Now and future



川上浩司
Koji KAWAKAMI
京都大学大学院医学研究科薬剤疫学

◎一口に臨床研究といっても，そのめざすところは多様である．臨床研究には，新医療開発のためのトランスレーショナル研究，臨床現場におけるクリニカルクエスチョンに仮説を立てて検証し，ひいてはエビデンスに基づく医療を実践するための臨床疫学研究，薬剤疫学などの範疇で，医療や薬剤の費用対効果を測定する比較効用性研究，患者個人に対する治療の選定にもかかる患者中心アウトカム研究など，すべてが重要な臨床研究の領域となっている．本稿では，これらについて世界および日本の動向を概説する．

Key word：臨床疫学，薬剤疫学，トランスレーショナルリサーチ，ヘルステクノロジーアセスメント，比較効用性研究

臨床研究における3つの柱

臨床研究には，①新規の医薬品や医療機器の開発にかかる臨床試験，すなわち，トランスレーショナル研究といわれる探索的臨床試験や医薬品の大規模な検証的臨床試験，②臨床現場における自らの診療上の疑問を明らかにするために観察研究や介入研究のデザインで研究を実施し，その結果を診療ガイドラインの改訂に反映させてエビデンスに基づいた医療(evidence-based medicine：EBM)へとつなげるための臨床疫学研究，そして，③診療情報，支払情報，調剤情報，生体情報などのデータベースを利・活用し，薬剤疫学や臨床疫学の手法で観察研究を行い，費用対効果を含む比較効用性研究なども踏まえてあらたな知見を得て社会における医療の受容を勘案し，また基礎研究の道しるべともするための臨床研究といった領域が存在する(図1)．

本稿では，日本における臨床研究の現状をこれらの領域別に考察し，将来に向けて必要な整備について提案する．

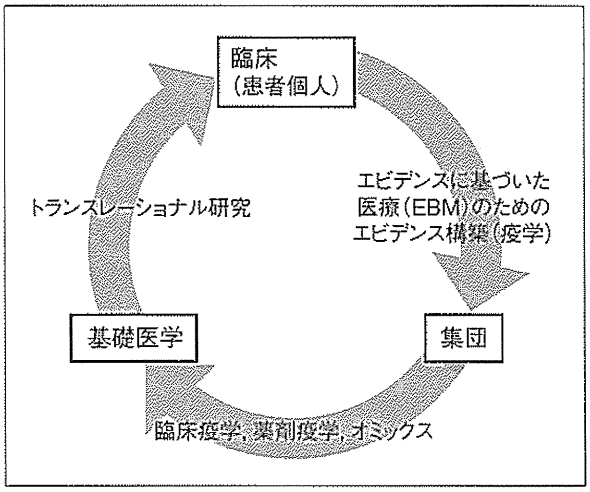


図1　臨床研究のサイクル

医薬品や医療機器開発にかかる臨床試験

現在の日本の薬事法は，医薬品などを繰り返して製造し，国内において販売・流通させるという製造販売業を規制している．それゆえ，規制の対象は大学等研究機関ではなく，営利企業(製薬企業)となっている．薬事法の規定内で，国(厚生労働大臣)からの承認を受けることを目的とした臨床試験は“治験”とよばれており，承認後は薬価収載されて国内の医療機関での当該医薬品の使用が可能となる．この場合，臨床試験(治験)の実施，および治験終了後には，独立行政法人医薬品医療